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Xanthohumol in XERME®, a xanthohumol-enriched roasted malt extract, and protection of DNA from oxidative damage

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Xanthohumol in XERME[®], a xanthohumol-enriched roasted malt extract, and protection of DNA from oxidative damage: evaluation of a health claim pursuant to Article 13(5) of Regulation (EC) No 1924/2006

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA),
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Peter Willatts, Ambroise Martin, Sean (JJ)Strain and Alfonso Siani

Abstract

Following an application from TA-XAN AG, submitted for authorisation of a health claim pursuant to Article 13(5) of Regulation (EC) No 1924/2006 via the Competent Authority of Germany, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver an opinion on the scientific substantiation of a health claim related to xanthohumol in XERME[®], a xanthohumol-enriched roasted malt extract, and protection of DNA from oxidative damage. The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence. The food proposed by the applicant as the subject of the health claim is xanthohumol in XERME[®], a xanthohumol-enriched roasted malt extract. The Panel considers that xanthohumol in XERME[®], a xanthohumol-enriched roasted malt extract, is sufficiently characterised. The claimed effect proposed by the applicant is 'helps to maintain the integrity of DNA and protects against oxidative damage in the cells of the body'. The Panel considers that protection of DNA from oxidative damage is a beneficial physiological effect. The only human study from which conclusions can be drawn for the scientific substantiation of the claim did not show an effect of xanthohumol in XERME[®] on the protection of DNA from oxidative damage. In the absence of evidence for an effect of xanthohumol in XERME[®] on the protection of DNA from oxidative damage, the studies provided by the applicant on the proposed mechanisms by which the food/constituent could exert the claimed effect were not considered by the Panel for the scientific substantiation of the claim. The Panel concludes that a cause and effect relationship has not been established between the consumption of xanthohumol in XERME[®], a xanthohumol-enriched roasted malt extract, and protection of DNA from oxidative damage.

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Keywords: xanthohumol, XERME[®], DNA integrity, oxidative damage, health claim

Requestor: Competent Authority of Germany following an application by TA-XAN AG

Question number: EFSA-Q-2017-00663

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Summary

Following an application from TA-XAN AG, submitted for authorisation of a health claim pursuant to Article 13(5) of Regulation (EC) No 1924/2006 via the Competent Authority of Germany, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver an opinion on the scientific substantiation of a health claim related to xanthohumol in XERME®, a xanthohumol-enriched roasted malt extract, and protection of DNA from oxidative damage.

The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence.

The general approach of the NDA Panel for the evaluation of health claims applications is outlined in the EFSA general guidance for stakeholders on health claim applications and the guidance on the scientific requirements for health claims related to antioxidants, oxidative damage and cardiovascular health.

The food proposed by the applicant as the subject of the health claim is 'xanthohumol presented as xanthohumol-enriched roasted malt extract (XERME®)'. The Panel considers that xanthohumol in XERME®, a xanthohumol-enriched roasted malt extract, which is the subject of the health claim, is sufficiently characterised.

The claimed effect proposed by the applicant is 'helps to maintain the integrity of DNA and protects against oxidative damage in the cells of the body'. The proposed target population is 'adults in the general population'. The Panel considers that protection of DNA from oxidative damage is a beneficial physiological effect.

The only human study from which conclusions can be drawn for the scientific substantiation of the claim did not show an effect of xanthohumol in XERME® on the protection of DNA from oxidative damage.

In the absence of evidence for an effect of xanthohumol in XERME® on the protection of DNA from oxidative damage, the studies provided by the applicant on the proposed mechanisms by which the food/constituent could exert the claimed effect were not considered by the Panel for the scientific substantiation of the claim.

On the basis of the data provided, the Panel concludes that a cause and effect relationship has not been established between the consumption of xanthohumol in XERME®, a xanthohumol-enriched roasted malt extract, and protection of DNA from oxidative damage.

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

Regulation (EC) No 1924/2006¹ harmonises the provisions that relate to nutrition and health claims, and establishes rules governing the Community authorisation of health claims made on foods. As a rule, health claims are prohibited unless they comply with the general and specific requirements of this Regulation, are authorised in accordance with this Regulation, and are included in the lists of authorised claims provided for in Articles 13 and 14 thereof. In particular, Article 13(5) of this Regulation lays down provisions for the addition of claims (other than those referring to the reduction of disease risk and to children's development and health) which are based on newly developed scientific evidence, or which include a request for the protection of proprietary data, to the Community list of permitted claims referred to in Article 13(3).

According to Article 18 of this Regulation, an application for inclusion in the Community list of permitted claims referred to in Article 13(3) shall be submitted by the applicant to the national competent authority of a Member State, which will make the application and any supplementary information supplied by the applicant available to the European Food Safety Authority (EFSA).

1.2. Interpretation of the Terms of Reference

EFSA is requested to evaluate the scientific data submitted by the applicant in accordance with Article 16(3) of Regulation (EC) No 1924/2006. On the basis of that evaluation, EFSA will issue an opinion on the scientific substantiation of a health claim related to: xanthohumol in XERME®, a xanthohumol-enriched roasted malt extract, and protection of DNA from oxidative damage.

The present opinion does not constitute, and cannot be construed as, an authorisation for the marketing of xanthohumol in XERME®, a xanthohumol-enriched roasted malt extract, a positive assessment of their safety, nor a decision on whether xanthohumol in XERME®, a xanthohumol-enriched roasted malt extract, is, or is not, classified as a foodstuff. It should be noted that such an assessment is not foreseen in the framework of Regulation (EC) No 1924/2006.

It should also be highlighted that the scope, the proposed wording of the claim, and the conditions of use as proposed by the applicant may be subject to changes, pending the outcome of the authorisation procedure foreseen in Article 18(4) of Regulation (EC) No 1924/2006.

2. Data and methodologies

2.1. Data

Information provided by the applicant

Food/constituent as stated by the applicant

According to the applicant, the food for which the health claim is made is 'xanthohumol, presented as xanthohumol enriched roasted malt extract (XERME). Xanthohumol is considered to be the bioactive ingredient in this product. It is uniquely presented, in the TA-XAN product (XERME), in combination with a roasted malt extract to enhance solubility of the xanthohumol and to stabilize the xanthohumol by suppressing the isomerization of xanthohumol to iso-xanthohumol. Thus it is believed that the roasted malt extract thereby enhances the oral bioavailability of xanthohumol. The roasted malt extract is a delivery vehicle and it is xanthohumol which remains the bioactive ingredient. Xanthohumol enriched roasted malt extract (XERME), [is] a product that can be added to other foods, beverages and dietary supplements as a functional food ingredient'.

Health relationship as claimed by the applicant

According to the applicant, the claimed effect relates to: 'helps to maintain the integrity of DNA and protects against oxidative damage in the cells of the body. Damaged DNA is a widely-used biomarker of oxidative stress and is determined by single-cell gel electrophoresis (SCGE), commonly known as comet assay'.

¹ Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. OJ L 404, 30.12.2006, p. 9–25.

Mechanism by which the food/constituent could exert the claimed effect as proposed by the applicant

The applicant claims that 'DNA damage prevention was through its modulation of various factors such as induction of the Phase II enzymes like QR, which is achieved through covalently modifying Keap 1 protein. In cancerous cells, this enzyme was inhibited by xanthohumol. Additionally, other enzymes like Nrf2, an emerging modulator of cellular resistance to oxidants, were also activated by xanthohumol to confer cytoprotection. Xanthohumol's inhibition of pro-carcinogens was also attributed to its inhibition of CYP1A activity in a concentration-dependent manner and not through its antigenotoxic action. The prevention of oxidative DNA damage was nevertheless not only associated with xanthohumol's free radicals scavenging trait as xanthohumol was shown to be a moderate free radical quencher. Xanthohumol showed highly selective effects in dysregulated systems as opposed to normal cells where growth suppression and apoptotic events were more pronounced in the abnormal cells and little or no effect was reported in healthy cells'.

Wording of the health claim as proposed by the applicant

The applicant has proposed the following wording for the health claim: 'helps to maintain the integrity of DNA and protects against oxidative damage in the cells of the body'.

Specific conditions of use as proposed by the applicant

According to the applicant, the target population for the intended health claim is 'healthy adults over 18 years of age who wish to maintain their overall health status'. 'Daily consumption of 12 mg of xanthohumol taken as a beverage containing the xanthohumol-enriched roasted malt extract (in up to 3 divided applications) has been shown to lead to a reduction in oxidative DNA damage'.

Data provided by the applicant

Health claim application on xanthohumol and pursuant to Article 13.5 of Regulation 1924/2006, presented in a common and structured format as outlined in the Scientific and technical guidance for the preparation and presentation of applications for authorisation of health claims.²

As outlined in the General guidance for stakeholders on health claim applications,³ it is the responsibility of the applicant to provide the totality of the available evidence.

2.2. Methodologies

The general approach of the NDA Panel for the evaluation of health claims applications is outlined in the EFSA general guidance for stakeholders on health claim applications (EFSA NDA Panel, 2016).

The scientific requirements for health claims related to antioxidants, oxidative damage and cardiovascular health are outlined in a specific EFSA guidance (EFSA NDA Panel, 2018).

The application contains data claimed as confidential: starting materials, specifications, product analysis, manufacturing process and information on stability and bioavailability of XERME product and study report for the unpublished human intervention study (specified in details in Confidentiality Decision).

3. Assessment

3.1. Characterisation of the food/constituent

The food proposed by the applicant as the subject of the health claim is 'xanthohumol, presented as xanthohumol-enriched roasted malt extract (XERME®)'.

Xanthohumol (1-(2,4-dihydroxy-6-methoxy-3-(3-methylbut-2-en-1-yl)phenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one) is the principal prenylated flavonoid of the female inflorescences of the hop plant (*Humulus lupulus* L.), an ingredient of beer. It represents 0.1–1% of dry weight of hops.

Xanthohumol can be measured in foods by established methods.

The viscous liquid XERME® is a product containing xanthohumol (1.2–1.8 g/100 g) in combination with a roasted malt extract to enhance solubility and stabilise the xanthohumol by suppressing its

² EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA); Scientific and technical guidance for the preparation and presentation of an application for authorisation of a health claim (revision 1). EFSA Journal 2011;9(5):2170, 36 pp. <https://doi.org/10.2903/j.efsa.2011.2170>

³ EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2016. General scientific guidance for stakeholders on health claim applications. EFSA Journal 2016;14(1):4367, 38 pp. <https://doi.org/10.2903/j.efsa.2016.4367>

isomerisation to iso-xanthohumol (details of the manufacturing process were provided, claimed as confidential by the applicant).

The information related to stability and batch-to-batch variability was provided.

The applicant was requested by EFSA to clarify whether the food/constituent for which the claim is made is xanthohumol (flavonoid present in hop plant) from any source or rather the product XERME (xanthohumol-enriched roasted malt extract), taking into account the implications that the choice could have on the number and amount of human intervention studies which could be considered as pertinent to the claim. In reply, the applicant clarified that the food/constituent for which the claim is made is XERME® (xanthohumol-enriched roasted malt extract). The Panel notes, however, that the two human intervention studies provided for the substantiation of the claim have been designed to address the effect of xanthohumol in XERME®, rather than the effects of XERME® itself.

The Panel considers that xanthohumol in XERME®, a xanthohumol-enriched roasted malt extract, which is the subject of the health claim, is sufficiently characterised.

3.2. Relevance of the claimed effect to human health

The claimed effect proposed by the applicant is 'helps to maintain the integrity of DNA and protects against oxidative damage in the cells of the body'. The proposed target population is 'healthy adults over 18 years of age who wish to maintain their overall health status'.

Upon a request from EFSA, the applicant clarified that the claimed effect refers to protection of DNA from oxidative damage.

The scientific substantiation of health claims on the protection of body cells and molecules from oxidative damage, including photo-oxidative (UV-induced) damage, requires at least one appropriate marker of oxidative modification of the target molecule assessed *in vivo* in human studies, preferably in combination with other marker(s). These other markers of oxidative damage to molecules cannot be used alone for substantiation, either because they represent a result of two processes (oxidative damage and repair), because they suffer from technical limitations (interferences from other unrelated processes or substances), or both. However, they can provide supportive evidence for the scientific substantiation of these claims. Different markers of oxidative damage to molecules should preferably be determined in the same study, but their determination in similar studies could be acceptable on a case-by-case basis.

Direct measurements of oxidative damage to DNA (i.e. oxidised DNA bases) can be obtained *in vivo* by using modifications of the comet assay (e.g. performed with endonuclease III (ENDO III) to detect oxidised pyrimidines and with formamidopyrimidine DNA glycosylase (FPG) to remove oxidatively damaged purines). Although the assay provides no absolute values, it allows quantitative comparison with an appropriate control. This assay directly reflects DNA oxidative damage within cells when assessed, for example, in circulating lymphocytes.

Measures of DNA damage using the traditional comet assay (single-cell gel electrophoresis, (SCGE)), which detect DNA strand breaks by the appearance of tailing, are not specific for oxidative damage. Other variants of the comet assay determine resistance against oxidative modification using *ex vivo* pro-oxidant challenges. Neither of these measurements is appropriate for assessing *in vivo* oxidative damage to DNA.

Analyses of 8-hydroxy-2-deoxy-guanosine (8-oxo-dG) in blood (e.g. lymphocytes), tissue (e.g. skin) and urine have been used to assess oxidative damage to DNA. Free 8-OHdG results from oxidative damage and excision-repair; it may also result from oxidation of free bases or nucleotides, from oxidation of other nucleic acids, and from artefacts during sample work up. Urinary 8-OHdG does not directly reflect DNA oxidation within cells, but can be used in combination with direct measurements of oxidative damage to DNA if appropriate techniques are used for analysis (e.g. liquid chromatography (LC)) (EFSA NDA Panel, 2018).

The Panel considers that protection of DNA from oxidative damage is a beneficial physiological effect.

3.3. Scientific substantiation of the claimed effect

The applicant performed a literature search in PubMed with the following key words: "oxidative damage" OR "DNA damage" OR "DNA repair" OR "healthy" OR "normal" OR "patient" AND "xanthohumol" (Search String 1) OR "oxidative damage" OR "DNA damage" OR "DNA repair" OR "healthy" OR "normal" OR "patient" AND "hop extract" (Search String 2) OR "oxidative damage" OR "DNA damage" OR "DNA repair" OR "healthy" OR "normal" OR "patient" AND "humulus lupulus" (Search String 3). The search was limited to trials published in English, between 1 January 1999 and 3 April 2017.

The applicant identified one published and one unpublished human intervention studies as being pertinent to the claim.

Ferk et al. (2016) in a randomised, cross-over, two-sequence, double-blind, placebo-controlled study evaluated the effect of xanthohumol in XERME® on DNA oxidative damage.

A total of 22 healthy individuals (11 females, mean age 25.0 ± 2.9 years) were enrolled and consumed daily either 1 L of a xanthohumol supplemented drink (containing 12.0 mg of xanthohumol, 1.0 g roast malt extract, 40.0 g sugar and 0.042 g malic acid) or an equivalent amount of xanthohumol-free placebo drink (with identical amounts of roasted malt extract, sugar, and malic acid) in the morning within 1 h for 14 consecutive days, followed by a 14-day wash-out period.

Blood samples were collected at baseline, during (at 4 h, 3 days, and 7 days), at the end of the intervention period (14 days), and after the wash-out. Twenty-four hour urine was collected at the start and at the end of the trial.

Sample size calculation was based on the assumption of a 24% difference in damaged DNA measured in peripheral lymphocytes in SCGE with three measurements and a SD of 56%, with 90% power and $\alpha = 0.05$ (Mišák et al., 2010). It was calculated that 20 subjects should be enrolled.

Oxidative damage to DNA, the primary outcome of the study, was assessed by using several methods: the comet assay (SCGE) with the standard protocol, the modified version of the comet assay (SCGE), using lesion-specific endonucleases (FPG and ENDO III) and after treatment with hydrogen peroxide. The measurements were performed in peripheral blood mononuclear cells (mostly lymphocytes) using a protocol previously described (Collins and Dusinska, 2002). These endpoints were determined as the difference of the comet tail intensities (% DNA in tail) between values obtained after 14 days of either treatment vs the respective baseline. The Panel notes that only two out of the four methods used, i.e. the modified comet assay performed with ENDO III and the FPG, directly reflect oxidative damage to DNA within cells.

Other outcomes evaluated in the study included the measurement of 8-oxo-dG and 8-oxo-guanosine (8-oxo-Guo) as markers of oxidative DNA degradation in urine, isoprostanes (15F2t-IsoP) in urine, oxidised low-density lipoprotein (oxLDL), serum malondialdehyde (MDA), ferric reducing ability of plasma (FRAP), serum C-reactive protein (CRP), serum 17β -oestradiol and progesterone, serum alkaline phosphatase (ALP), fasting glucose, serum cholesterol and triglyceride concentrations. Compliance with the treatment was verified through the measurement of xanthohumol concentrations in plasma.

Upon a request from EFSA, the applicant provided the statistical analysis plan for the study and statistical report. Statistical analyses were performed with analysis of variance (ANOVA) for dependent samples based on the means of the results obtained with three slides from each participant. A three factor mixed model ANOVA was applied with a sequence factor, a within-subjects time factor and a subject factor nested within the sequence factor. Post hoc comparisons between groups (xanthohumol in XERME® vs the xanthohumol-free placebo) were assessed by Dunnett's test.

All participants completed the study. No significant differences between groups were observed with respect to the modified version of the comet assay (SCGE) under standard conditions or after ENDO III treatment. In experiments with FPG, the extent of DNA migration after 14 days of xanthohumol intervention was significantly reduced as compared to the placebo, a significant treatment per time interaction was reported ($p < 0.001$). However, in the statistical report provided by the applicant, the effect was only observed in the study group who consumed xanthohumol first, but not in the group who consumed placebo first, with a significant sequence group difference ($p < 0.001$).

The Panel notes that a significant sequence effect was observed, which prevents drawing conclusions from this cross-over study. The Panel considers that no conclusion can be drawn from this study for the scientific substantiation of the claim.

The same publication reports the results of a one-arm, open-label study conducted in 10 subjects with xanthohumol capsules given for 14 days. The Panel notes that this study was not controlled and considers that no conclusions can be drawn from this study for the scientific substantiation of the claim.

Stevens, (2017) evaluated the effect of three doses of xanthohumol on oxidative damage to DNA in a randomised, single-centre, double-blind, cross-over study in healthy adults.

The test product was a non-alcoholic beverage (XERME®) fortified with either 2 or 8 mg of xanthohumol per bottle (0.33 L), so that daily doses of 6, 12 or 24 mg/day could be achieved by consuming three bottles per day. The placebo was a beverage with the same nutritional content and the same sensory characteristics as the test beverage but without xanthohumol.

Subjects were assigned to one of the three groups, in which each subject consumed one dose of xanthohumol (6, 12 or 24 mg/day) or the respective placebo for 3 weeks. After a 3-week washout period subjects switched to the alternative product (xanthohumol or placebo) for another three weeks. Subjects

were asked to consume 1 L of the beverage in three doses daily (i.e. 3×330 mL) at 08:00 a.m., 12:00 p.m. and 20:00 p.m. The methods used for randomisation were not specified in the study report.

Sample size was calculated based on pharmacokinetics data from previous studies with the assumption that approximately 22 subjects per dose arm would be sufficient to distinguish between the 6 and the 12 mg dose groups and between the 12 and the 24 mg dose groups regarding steady-state plasma levels of xanthohumol (power $\geq 80\%$ and type I error $< 5\%$). A total of 86 volunteers were enrolled; 17 withdrew voluntarily and 5 were removed owing to abnormal blood tests or not meeting inclusion/exclusion criteria. A total of 66 healthy volunteers were randomised ($n = 22$ per group).

The authors claim that the primary outcomes were 8-oxo-dG in urine, results from the comet assay (both standard and modified FPG) in peripheral blood mononuclear cells (PBMCs), 8-oxo-dG in plasma, and the ratio of reduced glutathione (GSH) to oxidised GSH in plasma. Both the intensity of the tail and the length of the tail, i.e. DNA tail moment, were assessed in the calculation of the results of the comet assay. However, the Panel notes that none of these variables was used to power the study. Other outcomes assessed were xanthohumol concentrations and its metabolites in plasma and, adverse events during the study.

Oxidative damage to DNA was evaluated by the standard comet assay both in PBMC and in whole blood, and the modified comet assay with FPG in lymphocytes. The Panel notes that only the modified comet assay with FPG in lymphocytes directly reflects oxidative damage to DNA within cells.

In statistical analysis, at each treatment dose (6, 12 and 24 mg/day), data were analysed as repeated measures in time design using general linear mixed model (PROC MIXED). Fixed effects in the model were treatment group, gender, age group (19–29 years, 30–39 years, 40–49 years), sampling time (before period 1, after period 1, before period 2, after period 2), and the interaction between treatment group and sampling time. Repeated measures within subjects were modelled using an unstructured variance-covariance structure. The 'change' in the xanthohumol group was compared statistically to the 'change' in the respective control group using parametric tests (ANOVA) and the difference between the two groups was set at statistically significance at $p < 0.05$. The Panel notes that the latter comparison is inappropriate for crossover study design. The results given below are based on the general linear mixed model.

All randomised subjects completed the study. However, two participants were removed from the final analysis of the study *a posteriori* after re-analysis of blood tests (due to abnormal results of liver tests).

There were no statistically significant differences between any xanthohumol dose tested and the respective control regarding oxidative DNA damage measured as either median tail intensity or median tail moment in lymphocytes using the modified comet assay in the presence of FPG or in PBMC using the standard alkaline comet assay.

A statistically significant reduction in median tail intensity measured by the standard comet assay in whole blood was observed in the 12 mg/day xanthohumol group compared to placebo (for xanthohumol: baseline 0.47 ± 0.06 , change -0.13 ± 0.08 , for placebo: baseline 0.31 ± 0.07 , change 0.17 ± 0.08 , $p = 0.01$) but not in median tail moment, or on either median tail intensity or median tail moment in the 6 mg/day or the 24 mg/day xanthohumol groups.

Urinary 8-oxo-Guo concentrations and blood concentrations of GSH were not significantly different between the xanthohumol and placebo groups at any dose. Blood concentration of oxidised GSH was significantly lower in the 6 mg/day xanthohumol group compared to placebo, but not in the 12 mg/day or the 24 mg/day groups.

The Panel notes that no effect of xanthohumol in XERME® was observed on DNA oxidative damage measured in lymphocytes by the modified comet assay in the presence of FPG, a direct measure of DNA oxidative damage within cells. The Panel also notes that the results of other non-specific tests used in this study to assess oxidative damage were either not statistically significant between the xanthohumol and placebo groups at any xanthohumol dose (i.e. standard alkaline comet assay in PBMC, urinary 8-oxo-Guo concentrations, blood concentrations of GSH), or inconsistent across xanthohumol doses (i.e. standard alkaline comet assay in whole blood, blood concentrations of oxidised GSH).

The Panel considers that this study does not show an effect of xanthohumol in XERME® on the protection of DNA from oxidative damage.

The Panel considers that the only human study from which conclusions can be drawn for the scientific substantiation of the claim did not show an effect of xanthohumol in XERME® on the protection of DNA from oxidative damage.

In the absence of evidence for an effect of xanthohumol in XERME® on the protection of DNA from oxidative damage, the studies provided by the applicant on the proposed mechanisms by which the food/constituent could exert the claimed effect were not considered by the Panel for the scientific substantiation of the claim.

Weighing of the evidence

In weighing the evidence, the Panel took into account that the only study from which conclusions can be drawn for the scientific substantiation of the claim (Stevens, 2017) did not show an effect of xanthohumol in XERME® on the protection of DNA from oxidative damage.

The Panel concludes that a cause and effect relationship has not been established between the consumption of xanthohumol in XERME®, a xanthohumol-enriched roasted malt extract, and protection of DNA from oxidative damage.

4. Conclusions

On the basis of the data presented, the Panel concludes that:

- the food/constituent, xanthohumol in XERME®, a xanthohumol-enriched roasted malt extract, which is the subject of the health claim, is sufficiently characterised.
- the claimed effect proposed by the applicant is 'helps to maintain the integrity of DNA and protects against oxidative damage in the cells of the body'. The target population proposed by the applicant is 'healthy adults over 18 years of age who wish to maintain their overall health status'. Protection of DNA from oxidative damage is a beneficial physiological effect.
- A cause and effect relationship has not been established between the consumption of xanthohumol in XERME®, a xanthohumol-enriched roasted malt extract, and protection of DNA from oxidative damage.

Steps taken by EFSA

Health claim application on 'xanthohumol' and 'protection of DNA from oxidative damage' pursuant to Article 13(5) of Regulation (EC) No 1924/2006 (Claim serial No: 0463_DE). Submitted by TA-XAN AG, Adolfsallee 21 65185 Wiesbaden, Germany.

- 1) This application was received by EFSA on 12/9/2017.
- 2) The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence.
- 3) The scientific evaluation procedure started on 14/11/2017.
- 4) On 16/11/2017, the Working Group on Claims of the NDA Panel agreed on a list of questions for the applicant to provide additional information to accompany the application. The scientific evaluation was suspended on 27/11/2017 and was restarted on 12/12/2017, in compliance with Article 18(3) of Regulation (EC) No 1924/2006.
- 5) During its meeting on 7/2/2018, the NDA Panel, having evaluated the data, adopted an opinion on the scientific substantiation of a health claim related to xanthohumol in XERME®, a xanthohumol-enriched roasted malt extract, and protection of DNA from oxidative damage.

References

- Collins A and Dusinska M. In: Armstrong D (ed.), 2002. *Oxidative Stress Biomarkers and Antioxidant Protocols*, Humana Press Inc., Totowa, New Jersey. pp. 147–160.
- EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2016. General scientific guidance for stakeholders on health claim applications. EFSA Journal 2016;14(1):4367, 38 pp. <https://doi.org/10.2903/j.efsa.2016.4367>
- EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), Turck D, Bresson J-L, Burlingame B, Dean T, Fairweather-Tait S, Heinonen M, Hirsch-Ernst K-I, Mangelsdorf I, McArdle HJ, Naska A, Neuhäuser-Berthold M, Nowicka G, Pentieva K, Sanz Y, Sjödin A, Stern M, Tomé D, Van Loveren H, Vinceti M, Willatts P, Martin A, Strain JJ, Heng L, Valtueña Martínez S and Siani A, 2018. Guidance for the scientific requirements for health claims related to antioxidants, oxidative damage and cardiovascular health (revision 1). EFSA Journal 2018;16(1):5136, 21 pp. <https://doi.org/10.2903/j.efsa.2018.5136>
- Ferk F, Mišík M, Nersesyan A, Pichler C, Jäger W, Szekeres T, Marculescu R, Poulsen HE, Henriksen T, Bono R, Romanazzi V, Al-Serori H, Biendl M, Wagner KH, Kundi M and Knasmüller S, 2016. Impact of xanthohumol (a prenylated flavonoid from hops) on DNA stability and other health-related biochemical parameters: results of human intervention trials. Molecular Nutrition and Food Research, 60, 773–786.
- Mišík M, Hoelzl C, Wagner KH, Cavin C, Moser B, Kundi M, Simic T, Elbling L, Kager N, Ferk F, Ehrlich V, Nersesyan A, Dušinská M, Schilter B and Knasmüller S, 2010. Impact of paper filtered coffee on oxidative DNA-damage: results of a clinical trial. Mutation Research, 692, 42–48.

Stevens JF, Unpublished study report, 2017. Prevention of oxidative DNA damage by xanthohumol: a single-center, randomized, double-blind, cross-over study in healthy adults to evaluate the effects to dose and steady-state levels of xanthohumol and its major metabolites, isoxanthohumol and 8-prenylnaringenin.

Abbreviations

8-oxo-dG	8-hydroxy-2-deoxy-guanosine
8-oxo-Guo	8-oxo-guanosine
ALP	alkaline phosphatase
ANOVA	analysis of variance
CRP	C-reactive protein
ENDO	III endonuclease III
FPG	formamidopyrimidine glycosylase
FRAP	ferric reducing ability of plasma
GSH	glutathione
LC	liquid chromatography
MDA	malondialdehyde
NDA	EFSA Panel on Dietetic Products, Nutrition and Allergies
oxLDL	oxidised low-density lipoprotein
PBMC	peripheral blood mononuclear cells
QR	quinone reductase
SCGE	single-cell gel electrophoresis
UV	ultraviolet